Paternal imprinting of *dosage-effect defective1* contributes to seed weight xenia in maize

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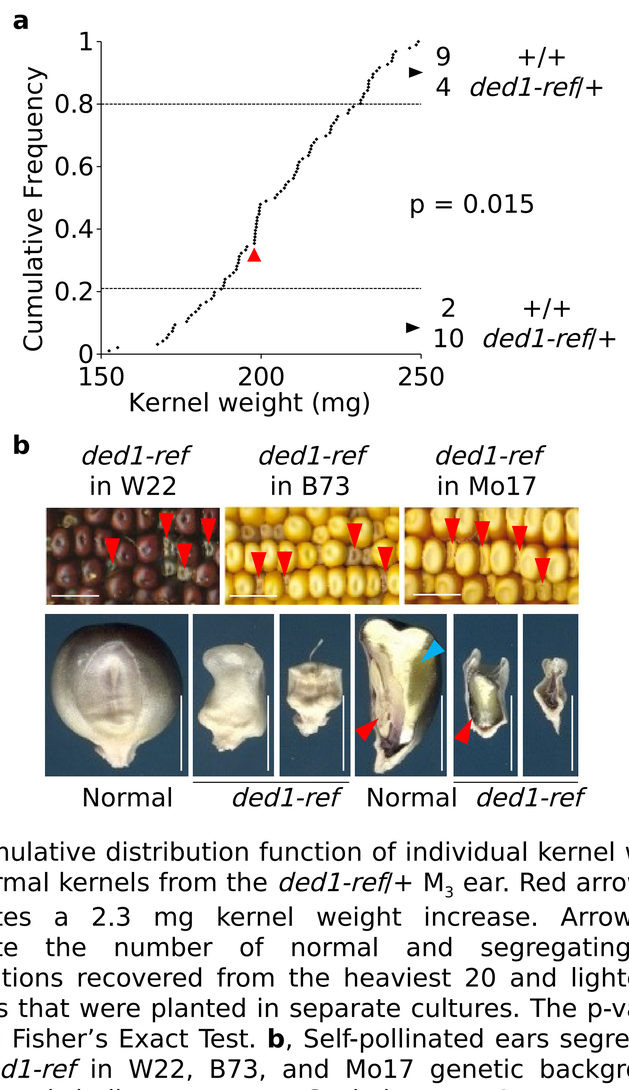
Abstract

Historically, xenia effects were hypothesized to be unique genetic contributions of pollen to seed phenotype, but most examples represent standard complementation of Mendelian traits. We identified the imprinted *dosage-effect defective1* (*ded1*) locus in maize (*Zea mays*) as a paternal regulator of seed size and development. Hypomorphic alleles show a quantitative seed weight reduction when *ded1* is transmitted through the male. *Ded1* encodes an R2R3-MYB transcription factor expressed specifically during early endosperm development with paternal allele bias. DED1 directly activates early endosperm genes and endosperm adjacent to scutellum cell layer genes, while directly repressing late grain-fill genes. These results demonstrate xenia as originally defined: Imprinting of *Ded1* causes the paternal allele to set the pace of endosperm development thereby influencing grain set and size.

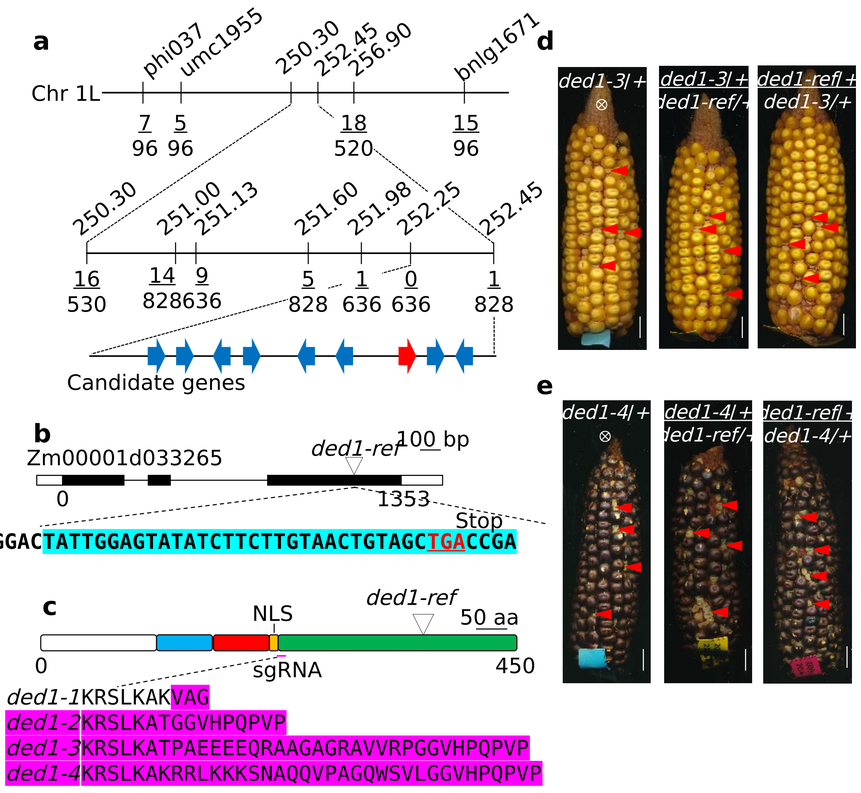
Results

Ded1 is a seed weight dosage effect gene



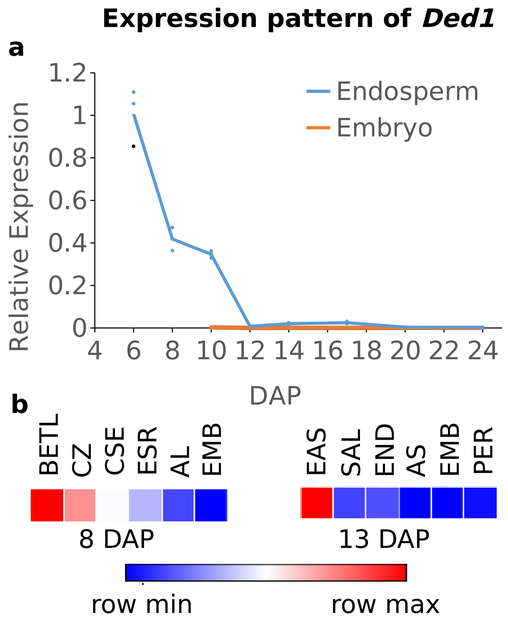
a, Cumulative distribution function of individual kernel weight for normal kernels from the *ded1-ref*/+ M_3 ear. Red arrowhead indicates a 2.3 mg kernel weight increase. Arrowheads indicate the number of normal and segregating self-pollinations recovered from the heaviest 20 and lightest 20 kernels that were planted in separate cultures. The p-value is from a Fisher's Exact Test. **b**, Self-pollinated ears segregating for *ded1-ref* in W22, B73, and Mo17 genetic backgrounds. Arrowheads indicate mutants. Scale bars are 1 cm.

Cloning of *Ded1*



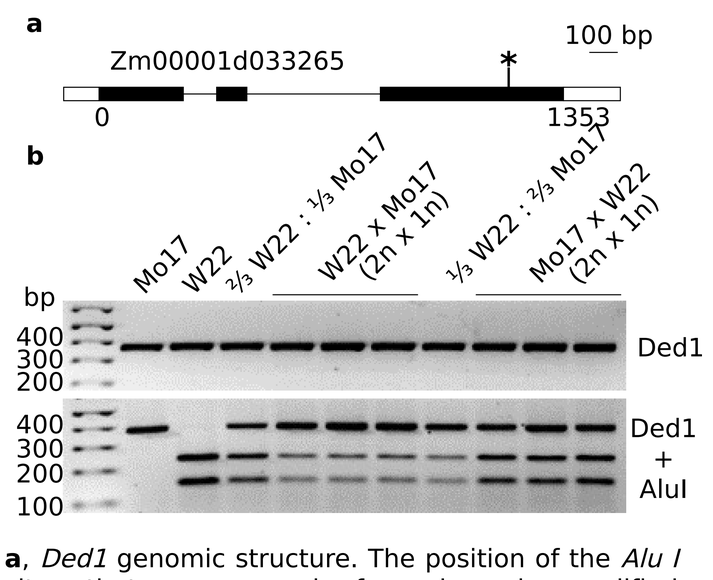
a, Fine mapping of the *ded1* locus. Molecular markers are indicated above physical maps and observed recombinants/genotyped progeny for each marker are below physical maps. Gene models in the fine-map interval are denoted with blue arrows with the *ded1* locus in red. **b**, Schematic of the B73_v4 genome annotation for the *Ded1* gene. Boxes are exons with coding sequences in black. Lines are introns. The triangle indicates the *ded1-ref* retrotransposon insertion. The 5' transposon junction sequence is highlighted in blue. **c**, Schematic of DED1 protein domains showing R2 (blue) and R3 (red) MYB DNA binding domains, the nuclear localization signal (yellow), and C-terminal acidic domain (green). The triangle indicates the *ded1-ref* insertion. Protein sequences of the Cas9-induced frameshift are highlighted in fuchsia. **d** and **e**, Self-pollination of *ded1-3*/+ (**d**) and *ded1-4*/+ (**e**) and reciprocal crosses with *ded1-ref*/+ in the B73 (**d**) and W22 (**e**) genetic background. Red arrowheads indicate mutant kernels

Expression pattern of *Ded1*



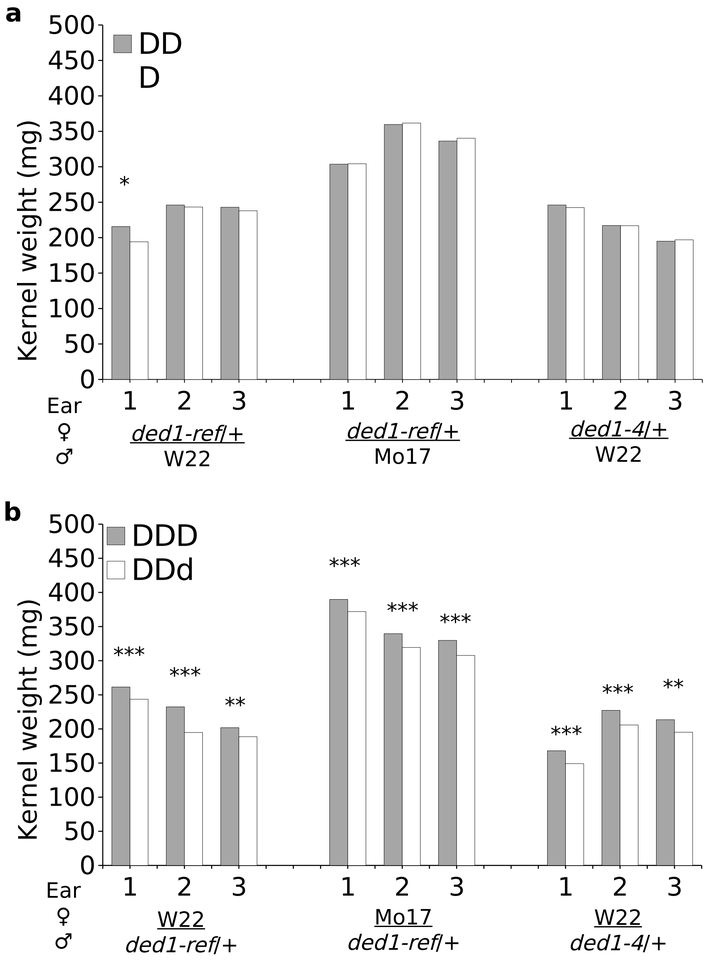
a. Expression of *Ded1* in dissected W22 endosperm and embryo tissues. Transcript levels were normalized to 18S rRNA. Data shown as mean ± SD (n = 3). **b**. Expression pattern of *Ded1* in various endosperm compartments based on reported transcriptome data. The gradient color scale indicates the relative expression level of each gene.

Paternal imprinting of the *ded1* locus



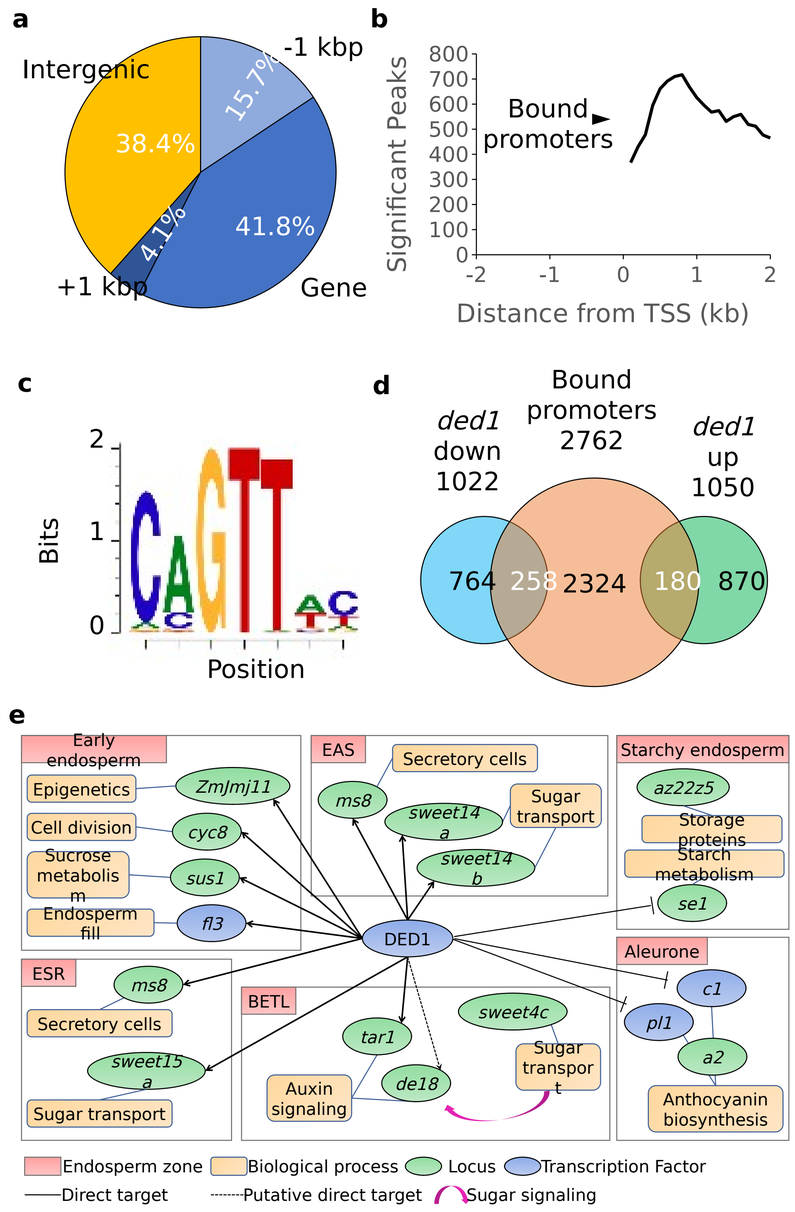
a, *Ded1* genomic structure. The position of the *Alu I* site that was used for cleaved amplified polymorphic sequence (CAPS) analysis (*Alu I* site present in W22 but absent in Mo17) is indicated by the asterisk. **b**, Allele-specific expression of *Ded1* in 12 DAP endosperm tissue comparing inbred self-pollinations and three biological replicates of reciprocal crosses between W22 and Mo17 with the female parent listed first. Proportional mixes of cDNA derived from W22 and Mo17 inbred are indicated with mix ratios. RT-PCR products were digested with *Alu*I to digest W22 products.

Paternal imprinting of *Ded1* contributes to seed weight



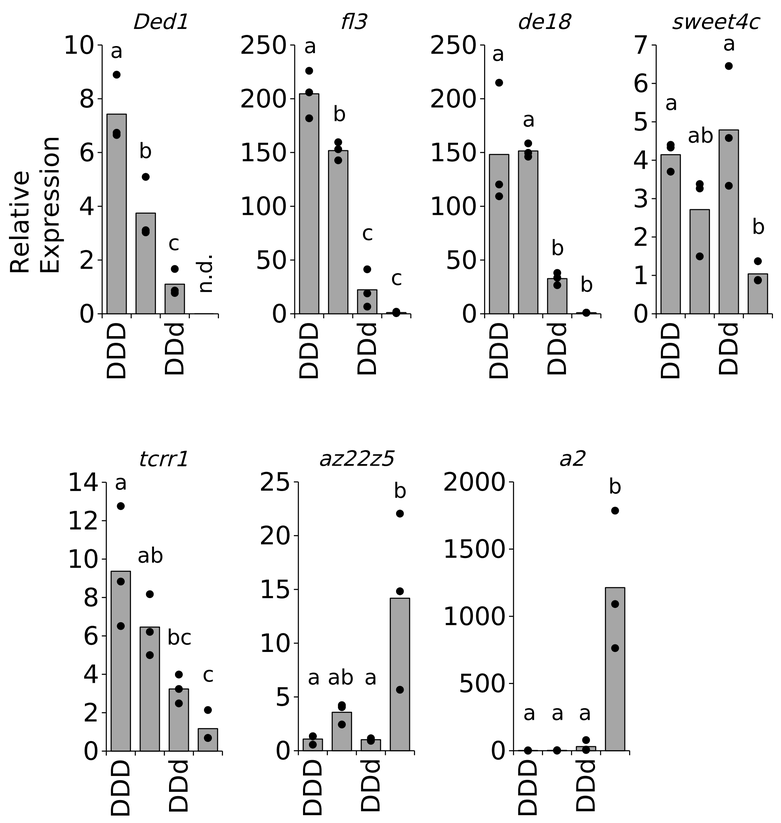
a and **b**, Average weights of homozygous normal and *ded1* heterozygous sibling kernels for maternal (**a**) and paternal (**b**) transmission of *ded1*. For each cross, the female parent is listed first and 90-96 kernels were scored from each of three ears. Data shown as mean ± SD. Student's t-test significance is indicated by * p<0.05, ** p<0.01, and *** p<0.001.

DED1 transcription factor targets



a, Distribution of DED1 DAP-seq peaks in the maize genome based on localization of peak summits. Genomic locations were defined as -1 kbp to the TSS; gene sequences from the TSS to TTS, 1 kbp downstream of the TTS; and intergenic regions. **b**, Number of DED1 DAP-seq peaks per 100 bp bin relative to annotated transcriptional start sites. Orange line shows the bound promoter region used to identify direct target genes. **c**, Sequence logo of top DED1 binding motifs identified by meme-chip v4.12.0 using the 5000 highest scoring DAP-seq peaks. **d**, Venn diagram showing genes bound by DED1 from -1 to +0.1 kb of the TSS that were tested for differential expression (orange) as well as DEGs from 12 DAP *ded1-ref* and normal sibling endosperm (q<0.05, FC>2, and TPM>1). Overlapping genes (white text) are inferred to be direct targets of DED1, including 258 DED1-activated and 180 DED1-repressed genes. **e,** Schematic of DED1 target loci with documented roles in endosperm development. DED1 activates loci that act early in endosperm development and in nutrient transfer tissues. DED1 represses loci acting later during grain fill.

Ded1 dosage effects on endosperm transcript levels



Expression of the normal allele of *Ded1* and DED1 downstream genes by qRT-PCR in four dosage states of *Ded1* (D) and *ded1-ref* (d). Genes analyzed included the *fl3* direct target, *de18* potential direct target, *sweet4c* activated DEG, *tcrr1* imprinted DEG, *az22z5* α-zein repressed DEG, and *a2* repressed DEG. Data points are averages of three technical replicates. Bars and error bars mean ± SD of three biological replicates (n = 3). Letters denote significant differences (P < 0.05) from Tukey's HSD test.